

Surface Functional Polymers by Post-Polymerization Modification using Diarylcarbenes: Introduction, Release and Regeneration of Hydrogen Peroxide and Bactericidal Activity

Jon-Paul Griffiths, Bushra Maliha,[†] Mark G. Moloney,* and Amber L. Thompson

Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford OX1 3TA, U.K.

Ishtiaq Hussain

Institute of Chemistry, University of Sargodha, Sargodha 40100, Pakistan.

[†] Visiting student from University of Punjab.

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Functionalized diarylcarbenes are excellent reactive intermediates suitable for the direct surface modification of organic polymers, and these may be used to introduce urea and thiourea functions onto polystyrene at loading levels of up to 2.3×10^{13} molecules/cm². These functions are capable of the reversible binding and release of peroxide at loading levels of up to 0.6 mmol/g and give polymers that display biocidal activity against a spectrum of gram-positive and gram-negative bacteria.

Introduction

The rapid rise of methicillin-resistant *Staphylococcus aureus* (golden staph) and other resistant bacteria has led to a significant increase in persistent bacterial infections in the human population worldwide;¹ this has proved to be a major problem in surgery, particularly where postoperative recovery is complicated by nosocomial infections acquired within the hospital environment. Typically these infections are treated with high doses of antibiotics, and this has ultimately led to the emergence of resistance in global bacterial populations.² The problem is not restricted solely to medical applications, being equally relevant to hygiene more generally, and the need to identify suitable solutions has been accelerated by the European Biocidal Products Directive (98/9/EC), which establishes a duty of care by European industries wherever possible to use antibacterial agents to protect the general population against harmful bacteria. There is current interest in the incorporation of biocides (which kill microorganisms) and biostats (which inhibit their growth) into diverse materials, illustrated by, for example, the development of antibacterial melamine resin surfaces.³ Moreover, recent reports indicate that antiseptic/antibacterial surfaces can be prepared by the incorporation of tetralkyl ammonium salts on glass surfaces⁴ or methylene blue and toluidine blue O onto silicone polymer or nanoparticle surfaces^{5,6} or by using a photodynamic

approach;⁷ furthermore, a biomimetic approach to biocidal activity, based on membrane disruption, would appear to be possible.⁸ Although there has also been considerable interest in the development of the antibacterial activity of silver-impregnated materials, there are concerns related to their nonspecific toxicity, especially in nanoparticulate form.⁹ The potential of halogenated furanones and isothiazolones in antifouling polymers has been demonstrated;¹⁰ when the organic antifouling agent was mixed with an extrudable polymer (preferably 1–10%), release rates of 0.1–100 $\mu\text{g}/\text{cm}^2/\text{day}$ of the biocidal agent conferred antifouling activity that lasted for extended periods of time (hundreds of days).

Perhaps the most general solution to the construction of biocidal polymers has been led by Sun¹¹ and Worley,^{12–14} who have developed immobilized *N*-halamines¹⁵ for applications in water purification¹⁶ and fabrics.^{17,18} However, the similar use of hydrogen peroxide, a metabolic oxidant ubiquitous in biological systems, has been less fully investigated. It has been known for some time that reactive oxygen species are capable of exerting antibacterial activity^{19,20} and antibodies produce hydrogen peroxide

*To whom correspondence should be addressed. E-mail: mark.moloney@chem.ox.ac.uk.

(1) Davies, D. *Nat. Rev. Drug Discovery* **2003**, *2*, 114–122.
(2) McBain, A. J.; Allison, D.; Gilbert, P. *Biotechnol. Genet. Eng. Rev.* **2000**, *17*, 267–279.
(3) Kandelbauer, A.; Widsten, P. *Prog. Org. Coat.* **2009**, *65*, 305–313.
(4) Tiller, H. C.; Liao, C.-J.; Lewis, K.; Klivanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5981–5985.
(5) Piccirillo, C.; Perni, S.; Gil-Thomas, J.; Prokopovich, P.; Wilson, M.; Pratten, J.; Parkin, I. P. *J. Mater. Chem.* **2009**, *19*, 6167–6171.
(6) Perni, S.; Piccirillo, C.; Pratten, J.; Prokopovich, P.; Chrzanowski, W.; Parkin, I. P.; Wilson, M. *Biomaterials* **2009**, *30*, 89–93.
(7) Rogers, S. A.; Krayner, M.; Lindsey, J. S.; Melander, C. *Org. Biomol. Chem.* **2009**, *7*, 603–606.

(8) Tew, G. N.; Lui, D.; Chen, B.; Doerksen, R. J.; Kaplan, J.; Carroll, P. J.; Klein, M. L.; Grado, W. F. D. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5110–5114.
(9) AshaRani, P. V.; Mun, G. L. K.; Hande, M. P.; Valiyaveetil, S. *ACS Nano* **2009**, *3*, 279–290.
(10) Christie, G. B.; Christov, V.; Nys, R. d.; Steinberg, P. D.; Hodson, S. *AU98/00509*, 1998.
(11) Sun, G.; Williams, J. F. *Chem. Ind.* **1999**, 658–661.
(12) Worley, S. D.; Sun, G. *Trends Polym. Sci.* **1996**, *4*, 364–370.
(13) Eknoian, M. W.; Worley, S. D. *J. Bioact. Compat. Polym.* **1998**, *13*, 303–314.
(14) Elrod, D. B.; Worley, S. D. *J. Bioact. Compat. Polym.* **1999**, *14*, 258–269.
(15) Worley, S. D.; Sun, G.; Sun, W.; Chen, T.-Y. U.S. Patent 5,490,983, 1996.
(16) Sun, G.; Allen, L. C.; Luckie, E. P.; Wheatley, W. B.; Worley, S. D. *Ind. Eng. Chem. Res.* **1995**, *34*, 4106–4109.
(17) Sun, G.; Xu, X. *Text. Chem. Color.* **1999**, *31*, 31–35.
(18) Sun, G.; Xu, X. *Text. Chem. Color.* **1998**, *30*, 26–30.
(19) Hirayama, J.; Wagner, S. J.; Abe, H.; Ikebuchi, K.; Ikeda, H. *Biol. Pharm. Bull.* **2001**, *24*, 418–421.
(20) Beghetto, C.; Renken, C.; Eriksson, O.; Jori, G.; Bernardi, P.; Ricchelli, F. *Eur. J. Biochem.* **2000**, *267*, 5585–5592.

when irradiated in the presence of oxygen, exerting an immediate and effective bactericidal effect.^{21,22} Thus, a biocidal polymer that released hydrogen peroxide would mimic some natural antibacterial processes and might represent an alternative solution to the problem of the antifouling of material surfaces. In fact, stabilized forms of hydrogen peroxide, such as polymer-supported material,²³ are known, but more common are urea complexes,^{24,25} whose stability arises from an extensive network of hydrogen bonding.^{26–28} An X-ray crystallographic analysis of a urea hydrogen peroxide (UHP) 1:1 complex indicated that each urea molecule was H bonded to five HOOH molecules;^{26,27} more recently, theoretical studies on this complex found that there are two stable adducts involving NHC(O)/HOOH or NHC(O)/HOOH bonding.²⁸ This suggested that similar immobilization might be achieved if a suitable polymer could be created, and this has been successfully reported by Panarin's group.^{29,30} More recently, a significant development came with the report from Ahmed and co-workers, who described the preparation of biocidal polymers based on uramil and its azo dyes, which also rely upon the release of hydrogen peroxide.^{31,32}

We were interested to determine if it might be possible to incorporate hydrogen peroxide binding and release behavior into existing materials by a postpolymerization modification rather than by designing and synthesizing the desired polymer *ab initio*.³³ The construction of modified polystyrenes by a post-polymerization approach is well known,^{34,35} and methods are still evolving³⁶ but we have developed a potentially more general approach based upon the reaction of a wide variety of materials with substituted diaryldiazomethanes in which a transient carbene is generated; we have found this approach to be superior to one involving the use of tosyl hydrazones³⁷ or diazirines,^{38,39} both well-known carbene precursors. This approach has been used to introduce color,⁴⁰ fluorescence,⁴¹ biocompatibility,⁴² and payload

delivery⁴³ effects to a wide range of substrates,⁴⁴ with a loading of the diarylmethyl unit onto the polymer surface of some 3×10^{14} molecules $\cdot \text{cm}^{-2}$.⁴⁵ We were interested to apply this concept to the development of biocidal polymers by making use of the reversible binding properties of urea and thiourea residues with hydrogen peroxide. By controlling the extent of hydrogen bonding and therefore the loading of the modified polymer, we expected to be able to further attenuate or enhance the biocidal activity of modified polymers that are suitable for diverse applications ranging from hygiene (antibacterial surfaces) to medical devices (such as contact lenses and medical implants) and water treatment with biocidal polymers.^{46–53} We report here the results of our investigation.

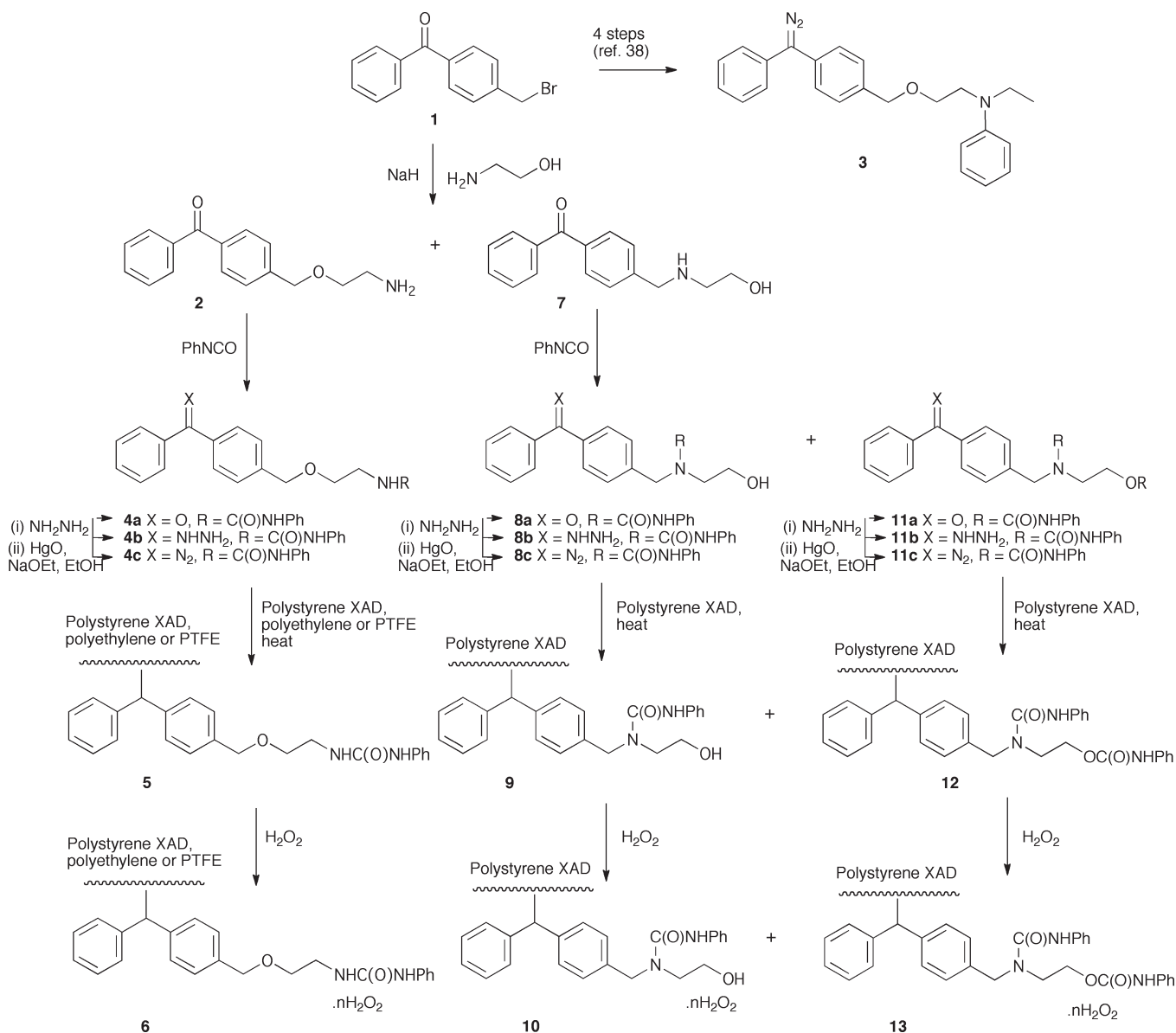
Results and Discussion

In the initial work, the required substituted benzophenones were found to be accessible by the reaction of the known bromomethylbenzophenone **1** with ethanolamine or (*N*-phenyl-*N*-ethyl)ethanolamine (Scheme 1) by analogy to a known procedure⁴⁰ to give amines **2** and **3**; a similar strategy has been reported for the synthesis of related substituted ethanolamines.⁵⁴ The location of the benzylic methylene singlet at a chemical shift of δ 4.65 in compound **2** was indicative of the ArCH_2O system, consistent with the displacement of the bromo substituent by the alkoxide function of the ethanolamine reagent. Direct treatment of **2** with phenylisocyanate gave urea **4a** in good overall yield,⁵⁵ which was readily distinguished by urea and ketone absorption signals in the IR spectrum at 1724 and 1658 cm^{-1} , respectively,⁵⁶ and by the expected NMR spectroscopic data. This material was converted to hydrazone **4b** and then diazo **4c**, whose structure was clearly indicated by IR absorbances at 2042 (diazo) and 1658 (urea) cm^{-1} . Diazo compound **4c** was used to treat several polymers (polystyrene XAD-4, polyethylene, PTFE) by adsorption of the diazo compound onto the polymer surface, followed by heating until the disappearance of the red color of the starting material, **4a**. Modified polymers **5** (polystyrene XAD-4, polyethylene, and PTFE) were treated with hydrogen peroxide to give the corresponding adducts **6**, for which an estimate of their loading was made by iodometric titration, and the antibacterial activity against both *S. aureus* and *E. coli* was demonstrated; the values obtained are given in Table 1. Blank polymer not treated with diazo compound **4c** displayed neither significant hydrogen peroxide uptake nor bioactivity. Of interest was that modified UHMWPE and PTFE materials **6** were inactive against *S. aureus*, and this suggested that the better activity of modified XAD-4 **6** was a result of its high surface area (750 m^2/g), which enabled significantly higher levels of peroxide loading. The stability of the

- (21) Lerner, R. A. *Science* **2001**, 293, 1806.
- (22) Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, 97, 10930.
- (23) Pourali, A. R.; Mersieh, G. *Bull. Korean Chem. Soc.* **2006**, 27, 1674–1676.
- (24) Taliansky, S. *Synlett.* **2005**, 1962–1963.
- (25) Ball, M. C.; Massey, S. *Thermochim. Acta* **1995**, 261, 95–106.
- (26) Fritchie, C. J.; McMullan, R. K. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1981**, 37, 1086–1091.
- (27) Lu, C.-S.; Hughes, E. W.; Giguere, P. A. *J. Am. Chem. Soc.* **1941**, 63, 1507–1513.
- (28) Dobado, J. A.; Molina, J.; Portal, D. *J. Phys. Chem. A* **1998**, 102, 778–784.
- (29) Panarin, E. F.; Kalnins, K. K.; Pestov, D. V. *Eur. Polym. J.* **2001**, 37, 375–379.
- (30) Panarin, E. F.; Kalnins, K. K.; Azanova, V. J. *Polym. Sci., Part A* **2007**, 49, 275–283.
- (31) Ahmed, A. E. I.; Hay, J. N.; Bushell, M. E.; Wardell, J. N.; Cavalli, G. *React. Funct. Polym.* **2008**, 68, 248–260.
- (32) Ahmed, A. E. I.; Hay, J. N.; Bushell, M. E.; Wardell, J. N.; Cavalli, G. *React. Funct. Polym.* **2008**, 68, 1448–1458.
- (33) Goddard, J. M.; Hotchkiss, J. H. *Prog. Polym. Sci.* **2007**, 32, 698–725.
- (34) Zinck, P.; Bonnet, F.; Mortreux, A.; Visseaux, M. *Prog. Polym. Sci.* **2009**, 34, 369–392.
- (35) Singh, B.; Sharma, N. *Polym. Degrad. Stab.* **2007**, 92, 876–885.
- (36) Leibfarth, F. A.; Kang, M.; Ham, M.; Kim, J.; Campos, L. M.; Gupta, N.; Moon, B.; Hawker, C. J. *Nat. Chem.* **2010**, 2, 207–212.
- (37) Braybrook, D. R.; Moloney, M. G.; Osborn, H. M. I.; Norris, W. J. *J. Photochem. Photobiol., A* **1993**, 70, 171.
- (38) Bentz, E.; Gibson, H.; Hudson, C.; Moloney, M. G.; Seldon, D. A.; Wearmouth, E. S. *Synlett* **2006**, 247.
- (39) Baldwin, J. E.; Pratt, A. J.; Moloney, M. G. *Tetrahedron* **1987**, 43, 2565.
- (40) Awenat, K.; Davis, P. J.; Moloney, M. G.; Ebenezer, W. *Chem. Commun.* **2005**, 990–992.
- (41) Wang, H.; Griffiths, J.-P.; Egdel, R. G.; Moloney, M. G.; Foord, J. *Langmuir* **2008**, 24, 862–868.
- (42) Choong, C.; Griffiths, J.-P.; Moloney, M. G.; Triffitt, J.; Swallow, D. *React. Funct. Polym.* **2009**, 69, 77–85.
- (43) Luksirikul, P.; Ballesteros, B.; Tobias, G.; Moloney, M. G.; Green, M. L. H. *Carbon* **2010**, 48, 1912–1917.
- (44) Moloney, M. G. *J. Phys. D: Appl. Phys.* **2008**, 41, 174006.
- (45) Leonard, D.; Moloney, M. G.; Thompson, C. *Tetrahedron Lett.* **2009**, 50, 3499–3502.

- (46) Grunzinger, S. J.; Kurt, P.; Brunson, K. M.; Wood, L.; Ohman, D. E.; Wynne, K. J. *Polymer* **2007**, 48, 4653–4662.
- (47) Muthukumar, N.; Maruthamuthu, S.; Ilangoan, A.; Palaniswamy, N. *Surf. Interface Anal.* **2007**, 39, 533–541.
- (48) Liang, J.; Chen, Y. J.; Ren, X. H.; Wu, R.; Barnes, K.; Worley, S. D.; Broughton, R. M.; Cho, U.; Kocer, H.; Huang, T. S. *Ind. Eng. Chem. Res.* **2007**, 46, 6425–6429.
- (49) Liang, J.; Owens, J. R.; Huang, T. S.; Worley, S. D. *J. Appl. Polym. Sci.* **2006**, 101, 3448–3454.
- (50) Chen, Z. B.; Sun, Y. Y. *J. Polym. Sci., Part A-1: Polym. Chem.* **2005**, 43, 4089–4098.
- (51) Liang, J.; Wu, R.; Huang, T. S.; Worley, S. D. *J. Appl. Polym. Sci.* **2005**, 97, 1161–1166.
- (52) Chen, Y. J.; Worley, S. D.; Kim, J.; Wei, C. I.; Chen, T. Y.; Suess, J.; Kawai, H.; Williams, J. F. *Ind. Eng. Chem. Res.* **2003**, 42, 5715–5720.
- (53) Simoes, M.; Pereira, M. O.; Vieira, M. J. *Biofouling* **2003**, 19, 287–295.
- (54) Guire, P. E.; Anderson, A. B.; Amos, R. A.; Everson, T. P. U.S. Patent 6,465,525, 2002.
- (55) Jeon, S.; Park, D. H.; Lee, H. K.; Park, J. Y.; Kang, S. O.; Nam, K. C. *Bull. Korean Chem. Soc.* **2003**, 24, 1465–1469.
- (56) Hocker, J. J. *J. Appl. Polym. Sci.* **1980**, 25, 2879–2889.

Scheme 1



peroxide adduct of XAD-4 **6** over a period of 96 h was measured by iodometric titration (Table 2), and it was found that approximately half the activity was lost in this time. The demonstration that the introduction and discharge of the peroxide of modified polystyrene XAD-4 **6** was possible was made over seven cycles, as indicated by the starch iodide test. It was similarly possible to demonstrate the successful uptake of hydrogen peroxide by polystyrene XAD-4 **21** modified with diazo compound **3**, which also exhibited regenerable activity over seven cycles (Table 1). These preliminary results indicated that, although surface modification to introduce antibacterial activity was feasible, loading levels of peroxide were important for measurable function and that further optimization was required.

We therefore set about investigating in detail and optimizing the application of this concept on polystyrene beads, chosen for the ease with which surface analysis at high loading levels is possible and its ease of handling. When the reaction of bromomethyl **1** with ethanolamine and then phenylisocyanate as outlined above was conducted at scale, it was found to give a separable mixture of urethanes **4a** and **8a** and bisurethane **11a** in yields of 18, 24, and 55% respectively; firm assignment of the

structures of compounds **8a** and **11a** by NMR analysis was inconclusive, as a result of very similar chemical shift values for their benzylic methylene resonance (δ 4.82 and 4.87) and $\text{OCH}_2\text{CH}_2\text{N}$ (3.67, 4.35 and 3.67, 4.25) systems compared to those for isomer **4a** (δ 4.65, 3.39, and 3.58 respectively), and the confirmation of their structures was achieved unequivocally only by single-crystal X-ray diffraction studies (Figures 1 and 2).⁵⁷ The formation of two products **8a** and **11a** was unexpected because earlier work on a smaller scale had indicated the exclusive formation of **2** and then **4a** only,⁵⁸ and this was traced to the formation of both **2** and **7** in the initial reaction. This outcome is most likely to arise from incomplete deprotonation of the alcohol, leading to reaction at either of the oxygen or nitrogen centers; the unequivocal synthesis of amine **2** has been recently reported using a route involving the protection of the amine function.⁵⁹ The reaction of **4a**, **8a**, and **11a** with hydrazine hydrate gave the corresponding hydrazones **4b**, **8b**, and **11b**, as a mixture of syn and

(57) Sandor, E.; Wooster, W. A. *Br. J. Appl. Phys.* **1963**, *14*, 506.

(58) Griffiths, J.-P.; Moloney, M. G. PCT/GB2006/000139, filed on 17th Jan 2006.

(59) Tang, R.-R.; Zhu, J.-J.; Luo, Y.-M. *Synth. Commun.* **2006**, *36*, 421–427.

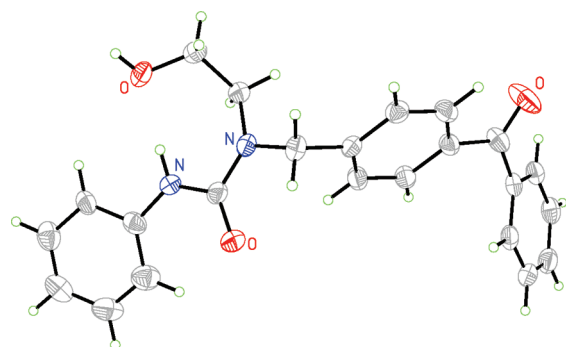
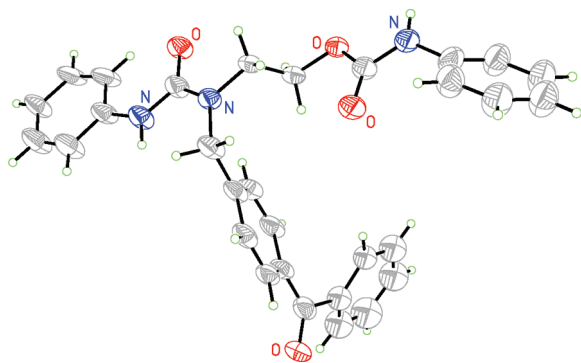
Table 1. Surface-Modified Polymers 6 and 21 (Schemes ¹ and ³)

modified polymer	polymer ^a	diazo compound	ratio ^b	starch iodide test ^c	loading of H ₂ O ₂ /mol g ^{-1f}	activity against <i>S. aureus</i> /mm ^g	activity against <i>E. coli</i> /mm ^g
6	XAD-4	4c	1:1	+	8.30×10^{-5d}	25	17
6	UHMWPE	4c	1:1	+	2.50×10^{-5}	inactive	—
6	PTFE	4c	1:1	+	1.07×10^{-5}	inactive	—
21	XAD-4	3	1:1	+	1.27×10^{-4e}	12	12

^a Polymers: Amberlite XAD-4 nonionic adsorbant (Aldrich 21,648-8); ultrahigh-molecular-weight polyethylene powder (Aldrich 43,426-4); polytetrafluoroethylene beads (Aldrich 18,247-8). ^b Weight/weight ratio of diazomethane 4c to polymer during functionalization step. ^c + = blue. ^d Average of three values. ^e Average of four values. ^f XAD-4, UHMWPE, and PTFE control samples all gave a negative starch iodide test; there was no measurable value for hydrogen peroxide loading and no observable bioactivity. ^g Zone of inhibition (diameter) results against *S. aureus* or *E. coli* using polymer (50 mg) in 10 mm wells in a hole-plate bioassay (against *Ceph C.* standard); (—) not determined.

Table 2. Stability of Hydrogen Peroxide-Loaded Polystyrene XAD-4 6 over Time

time interval/h	loading of hydrogen peroxide/mol g ⁻¹
0	8.42×10^{-5}
24	8.01×10^{-5}
48	9.61×10^{-5}
72	6.44×10^{-5}
96	4.13×10^{-5}

**Figure 1.** Crystal structure of 8a. Thermal ellipsoids are drawn at 50% probability, and the minor component of the disordered phenyl group is omitted for clarity.**Figure 2.** Crystal structure of 11a. Thermal ellipsoids are drawn at 50% probability, and the minor component of the disordered group is omitted for clarity.

anti isomers, which were used without further purification, and further reaction with alkaline mercuric oxide gave red-purple diazo products 8c and 11c in excellent yields; the formation of the diazo function was clearly indicated by the presence of an adsorption at 2040 cm⁻¹. In methanol, diazo products 8c and 11c undergo rapid decomposition, and in CDCl₃, they are stable for 1 to 3 h, making the procurement of spectroscopic data difficult; these solutions showed no trace of purple color after standing overnight. Decomposition was accelerated by light;

Table 3. Regenerability over Seven Cycles by the Charge/Discharge of Functionalized Polystyrene XAD-4 6 and 21, as Indicated by the Starch Iodide Test^a

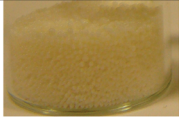
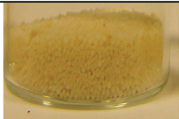

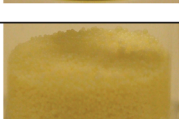
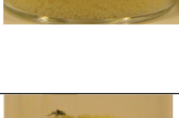

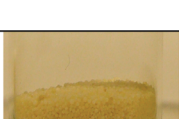
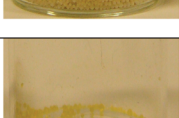
cycle	polystyrene XAD-4 6	polystyrene XAD-4 21
1	+	+
2	+	+
3	+	+
4	+	+
5	+	+
6	+	+
7	+	+

^a + = blue.

however, diaryldiazo compounds 4c, 8c, and 11c could be stored in neat form at -4 °C for 1 to 2 weeks without significant decomposition. Low pH also results in rapid decomposition of these diazo compounds. Adsorption onto polystyrene XAD, giving a red material, and then heating to 120 °C gave modified polymers 5, 9, and 12 in a reaction that proceeded with decoloration of the polymer. These materials were characterized by a combination of combustion analysis, ATR IR, and XPS analysis (Table 4). Unlike the diazo coupling of more conjugated systems,⁴⁵ the coupling in this case did not lead to intense coloration of the polymer, but the presence of carbonyl groups in the range of 1630–1720 cm⁻¹ and the ether functions at ca. 1200 cm⁻¹ were readily detected by ATR IR spectroscopy. This data is diagnostic because urea itself has IR signals at 1606, 1631, 1686, assigned to the amide I and II vibrations,⁶⁰ and reductions of this value of 60 cm⁻¹ in the crystalline form relative to solution have been reported.⁵⁶ Combustion analysis for the nitrogen content of modified polymers 5, 9, and 12 enabled the determination of the loading stoichiometry of the polymer (blank polymer shows no nitrogen by combustion analysis),^{42,45} and by assuming the manufacturer's value for the surface area of the polymer (750 m²/g), the surface loading for these two samples is 3.2×10^{12} and 5.6×10^{12} molecules·cm⁻². The increased loading level of 12 over 9, consistent with the formation of an additional carbamate function for the former, is noteworthy. These values are also consistent with loading levels in a related polystyrene system that is also modified by the diarylmethylene system and are determined to be 0.04–0.18 mmol/g or 2×10^{12} – 15×10^{12} molecules·cm⁻².⁴⁵ Confirmation of the presence of nitrogen and its loading density at the surface came from XPS analysis (Table 4). Both polystyrenes 9 and 12 showed the presence of nitrogen species from N 1s signals at binding energies (eV) of 400.2 and 400.3, respectively. (Oxygen O 1s signals at 532.2 and 531.8, respectively, arising from the desired surface modification as well as surface oxidation were also observed.) Moreover, the observed N/C ratios of 0.042 and 0.14 for 9 and 12, respectively, compare with the expected values of 0.084

(60) Stewart, J. E. *J. Chem. Phys.* **1957**, 26, 248–254.

Table 4. Characterization Data for Surface-Modified Polymers

Polymer	Appearance	ATR-IR (cm ⁻¹) ^a	Combustion Analysis/mmolg ⁻¹ (moleculescm ⁻²) ^b	XPS Analysis ^d
			[%areacoverage] ^c	
5		1720 (C=O) 1221 (C-O-C)	N, 0.28 (2.2 x 10 ¹³) [20.9]	C ₂₄ H ₂₆ O ₂ N ₂ Expected: N/C= 0.084 Found: N/C= 0.042
9		3250 (OH) 1713 (C=O) 1219 (C-O-C)	N, 0.035 (3.2 x 10 ¹²) [3.0]	C ₂₄ H ₂₆ O ₂ N ₂ Expected: N/C= 0.084 Found: N/C= 0.042
12		1657 (OC=O) 1631 (NC=O) 1219 (C-O-C)	N, 0.074 (5.6 x 10 ¹²) [5.3]	C ₃₁ H ₃₁ O ₃ N ₃ Expected: N/C= 0.097 Found: N/C= 0.14
15		1473 (C-N) 1213 (C=S) 1219 (C-O-C)	N, 0.28 S, <0.31 (2.2 x 10 ¹³) [20.9]	C ₂₄ H ₂₆ ON ₂ S Expected: N/C= 0.084, S/C= 0.042 Found: N/C= 0.076, S/C= 0.015
18		3565 (OH) 1445 (C-N and C=S) 1238 (C-O-C)	N, 0.26 S, <0.31 (2.0 x 10 ¹³) [19.0]	C ₂₄ H ₂₆ ON ₂ S Expected: N/C= 0.084, S/C= 0.042 Found: N/C= 0.073, S/C= 0.027
20		1219 (C-O-C)	N, 0.286 (2.3 x 10 ¹³) [20.9]	C ₂₅ H ₂₉ ON Expected: N/C= 0.042 Found: N/C= 0.022
23a		1712 (CO-NH- CO) 1220 (C-O-C)	N, 0.064 (5.2 x 10 ¹²) [4.9]	C ₂₉ H ₃₁ O ₅ N ₄ Expected: N/C= 0.18 Found: N/C= 0.04
23b		1711 (CO-NH- CO) 1445 (C=S) 1219 (C-O-C)	N, 0.10 S, <0.31 (8.0 x 10 ¹²) [7.6]	C ₂₉ H ₃₁ O ₃ N ₅ S Expected: N/C= 0.18, S/C= 0.034 Found: N/C= 0.087, S/C= 0.009

^a Blank polystyrene exhibited characteristic absorbances in the region of 2929, 1602, 1465, 1443, 989, 902 cm⁻¹, in keeping with literature data.⁶²

^b Calculated on the basis of 725 m²/g; ^c Assuming a limiting area of 1.44 nm²/molecule. (See the text for an explanation.) ^d Expected values calculated from molecular formulas assuming a surface monolayer of the carbene insertion product.

and 0.097, respectively, assuming the formation of a uniform monolayer of benzhydryl units with a molecular formula of C₂₄H₂₆O₂N₂ and C₃₁H₃₁O₃N₃ as required by the formulation of **9** and **12** in Scheme 1. These data may be further considered in

terms of the density of surface coverage of the layer: The limiting area (*A_o*) for the monolayer formation of benzene is 0.24 nm²/molecule, and that for substituted anthracenes is 0.45–0.48 nm²/molecule.⁶¹ By assuming a value for *A_o* of about 0.95 nm²/molecule for the substituted benzhydrylmethyl system derived from diazos **8c** and **11c**, materials **9** and **12** have surface loading densities of some 3.0% for **9** and up to 5.3% for **12**; this loading

(61) Birdi, K. S. *Self-Assembly Monolayer Structures of Lipids and Macromolecules at Interfaces*; Kluwer Academic/Plenum Publishers: New York, 1999.

Table 5. Loading Levels of Modified Polymers Calculated from Combustion and Iodometric Analysis

polymer	loading of N/S (molecules/cm ²)	N/S loading (mg/cm ²)	H ₂ O ₂ loading (mmol/g ⁻¹) ^a	H ₂ O ₂ loading (molecules/cm ²) ^a	H ₂ O ₂ loading (mg/cm ²) ^a
10	3.2×10^{12}	1.8×10^{-7}	1.18	4.3×10^{13}	2.4×10^{-6}
13	5.6×10^{12}	3.2×10^{-7}	0.50	9.6×10^{13}	5.4×10^{-6}
16	2.2×10^{13}	1.2×10^{-6}	1.05	1.29×10^{14}	7.3×10^{-6}
19	2×10^{13}	1.4×10^{-6}	0.47	6.4×10^{13}	3.6×10^{-6}
21	5.6×10^{12}	3.2×10^{-7}	0.16		
24a	5.2×10^{12}	2.9×10^{-7}	0.40	6.9×10^{13}	3.9×10^{-6}
24b	8×10^{12}	4.5×10^{-7}	0.63	1.18×10^{14}	6.6×10^{-6}

^a After 1 h of treatment with hydrogen peroxide of polymer beads.**Table 6. Stability Data of Hydrogen Peroxide-Loaded Modified Polymers**

time interval/h	loading of H ₂ O ₂ /mmol g ⁻¹ of 10	loading of H ₂ O ₂ /mmol g ⁻¹ of 13	loading of H ₂ O ₂ /mmol g ⁻¹ of 16	loading of H ₂ O ₂ /mmol g ⁻¹ of 19	loading of H ₂ O ₂ /mmol g ⁻¹ of 24a	loading of H ₂ O ₂ /mmol g ⁻¹ of 24b
0	1.20	0.54	1.61	0.80	0.86	1.47
24	1.00	0.45	0.88	0.59	0.76	1.05
48	0.78	0.36	0.86	0.45	0.75	1.02
72	0.75	0.33	0.85	0.50	0.73	0.97
96	0.50	0.35	0.80	0.50	0.71	0.89

Table 7. Summary of the Results of Blank and Modified Polymers

sample	N and S loading (mmol/g)		H ₂ O ₂ loading (mmol/g)		H ₂ O ₂ /N ratio		loss of activity after 96 h	bioassay results (zone of inhibition, mm)				
	N	S	1 h ^a	24 h ^b	1 h ^a	24 h ^b		<i>E. coli</i>	<i>S. aureus</i>	<i>B. thuringiensis</i>	<i>X. campestris</i>	<i>P. syringae</i>
blank	ND	ND	0.1	0.15	ND	ND	ND	6	6	4	5	11
6	0.28	ND						25	26	21	16	21
10	0.04	ND	0.50	0.54	12.5	13.5	58	25	28	18	12	16
13	0.07	ND	1.18	1.20	16	17	35	23	24	22	12	19
16	0.28	< 0.31	1.05	1.61	3.8	5.8	50	32	30	20	16	21
19	0.25	< 0.31	0.47	0.80	1.8	3.2	38	16	16	18	14	15
21	0.07	ND	0.15	0.19	2.1	2.7	ND	2	10	10	8	7
24a	0.06	ND	0.4	0.86	6.6	14	17	26	20	14	13	12
24b	0.10	< 0.31	0.63	1.47	6.3	15	39	28	29	22	17	20

^a After 1 h of immersion of polymer beads in H₂O₂. ^b After 24 h of immersion of polymer beads in H₂O₂.

density is in a range that is similar to values in a related system prepared by diarylmethylene insertion into polystyrene.⁴⁵

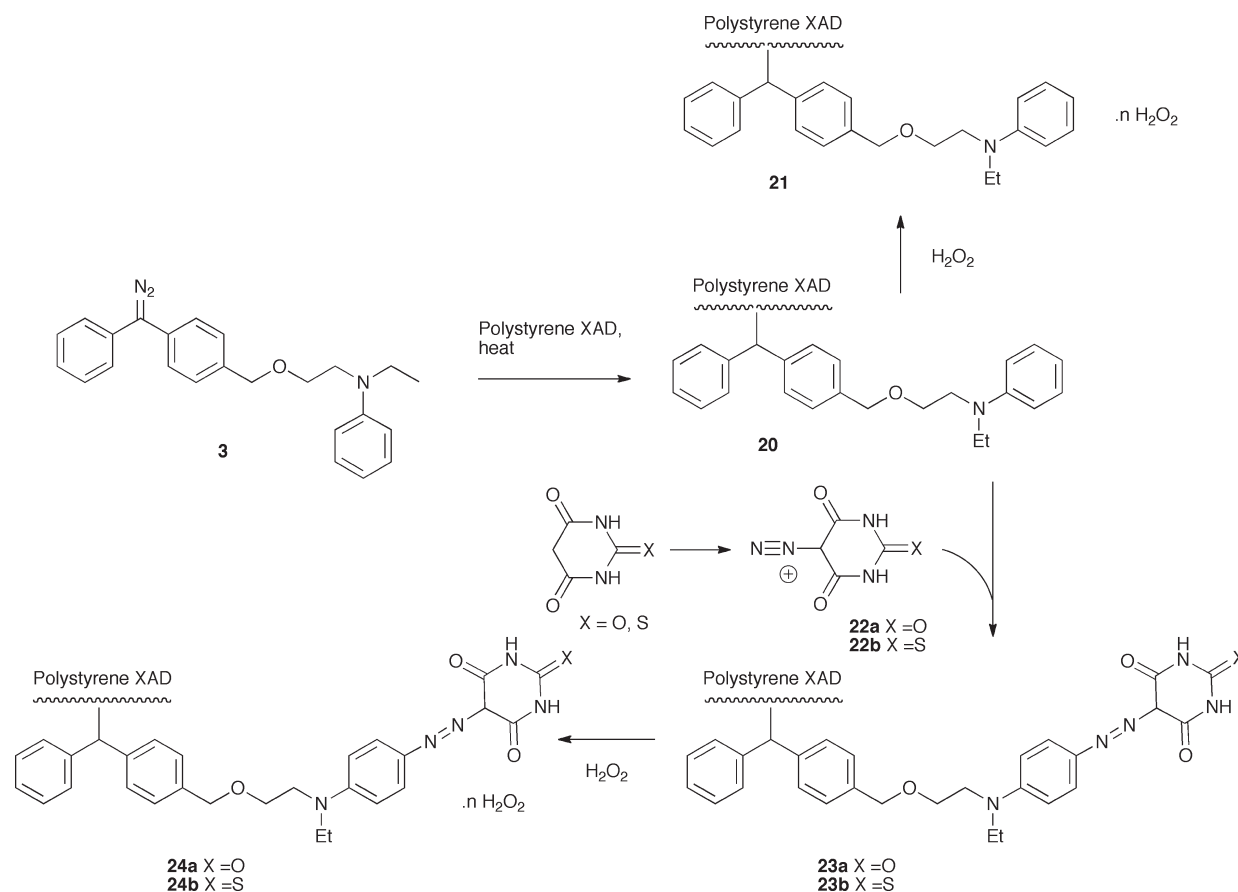
The treatment of modified polymers **5**, **9**, and **12** with hydrogen peroxide then gave polymers **10** and **13** in which the peroxide had been adsorbed, which was quantified by iodometric titration (Table 5) at levels of 4.3×10^{13} and 9.6×10^{13} molecules·cm⁻², in which polymer **13** displayed a loading that was approximately double that of **10**. Moreover, these polymers exhibited good stability, with their respective loadings decreasing to 42 and 65% of their initial values over 96 h (Table 6). The data in Tables 5 and 7 show that each urethane group in polymer **10** binds 12.5 molecules of H₂O₂ after 1 h of treatment with aqueous H₂O₂ (13.5 molecules after 24 h of treatment with aqueous H₂O₂) and each urethane group in polymer **13** binds 16 molecules after 1 h (17 after 24 h).

After the success of the phenyl isocyanate sequence (Scheme 1), the same reaction was exploited to introduce a thiourea function onto the polymer surface by reacting amine **2** and alcohol **7** with phenyl isothiocyanate (Scheme 2). This reaction gave a separable mixture of urethanes **14a** and **17a** (but not the product analogous to **11a**) in yields of 24 and 55%, respectively; the similarity of the NMR spectra of these two compounds did not allow the unequivocal structural assignment of these compounds, and a tentative assignment of the structure of **17a** was made on the basis of a higher downfield shift of the benzylic methylene relative to that of

14a (δ 5.32 vs δ 4.55) due to the immediately adjacent electron-withdrawing thiourea function. Unambiguous confirmation of the assignment of structure **17a** was achieved by single-crystal X-ray diffraction studies (Figure 3). Reacting **14a** and **17a** with hydrazine hydrate gave corresponding hydrazones **14b** and **17b** in yields of 89 and 73%, respectively, as a mixture of syn and anti isomers, which were used without further purification, and reacting **14a** and **17a** with manganese dioxide gave red or pink diazo product **14c** or **17c** in 91 or 89% yield, respectively; this oxidant was found to give better yields than the mercury oxide used with the urethanes above. The formation of the diazo function was clearly indicated by the presence of an adsorption at 2040 cm⁻¹ in the IR spectrum. These compounds were then reacted with polystyrene XAD-4 as described above, and products were **15** and **18** characterized by a combination of combustion analysis, ATR IR, and XPS analysis as indicated previously (Table 4). The presence of the thiocarbonyl group was readily detected at ca. 1450 cm⁻¹, and the ether function was detected in the region of 1220–1230 cm⁻¹ by ATR IR spectroscopy; thiourea is reported to exhibit strong signals at 1413 (C–S stretching vibration), 1473 (C–N bond stretching), and 1617 (NH₂ deformation).⁶⁰ Combustion analysis confirmed the presence of nitrogen and sulfur and gave surface loadings of 2.2×10^{13} and 2.0×10^{13} molecules·cm⁻² for polymers **15** and **18**, respectively. Similar loading levels, consistent with the presence of just one thiourea function in each polymer, are noteworthy in this case. These values are up to 10-fold higher than the loading levels in a related polystyrene system, which were determined to be 0.04–0.18

(62) Flores, K. O. V.; de Aguiar, A. P.; de Aguiar, M. R. M. P.; de Santa Maria, L. C. *Mater. Lett.* **2007**, *61*, 1190–1196.

Scheme 3



of nitrogen and sulfur in modified polymers **23a,b** and gave surface loadings for these two samples of 5.2×10^{12} and 8.0×10^{12} molecules $\cdot\text{cm}^{-2}$; the higher loading level of **23b** is noteworthy in this case. These values are consistent with loading levels in a related polystyrene system, which were determined to be 0.04–0.18 mmol/g or 2×10^{12} – 15×10^{12} molecules $\cdot\text{cm}^{-2}$.⁴⁵ Using a similar calculation with the limiting area (A_0) for monolayer formation assumed to be about 0.95 nm²/molecule for materials **15** and **18**, the surface loading densities are approximately 4.9 and 7.6%, respectively. This combustion data can also be used to estimate the yield of the diazo coupling step leading from polymer **20** to **23a,b** and was found to be 4.2 and 7.0%, respectively. XPS analysis (Table 4) of polymers **23a** and **23b** again confirmed the presence of nitrogen species from N 1s signals at binding energies (eV) of 400.7 and 400.2 and S 2p signals at binding energies (eV) of 169.3 and 164.7, respectively. The observed N/C ratios of 0.040 and 0.087 are somewhat lower than the expected value of 0.18, as is the observed S/C ratio of 0.0094 for thio derivative **23b** (expected value of 0.034), suggesting that something less than a uniform monolayer of benzhydryl units with molecular formulas of $\text{C}_{29}\text{H}_{31}\text{N}_5\text{O}_4$ and $\text{C}_{29}\text{H}_{31}\text{N}_5\text{O}_3\text{S}$ as required by the formulation of **23a** and **23b** in Scheme 1 is formed. This outcome is likely to be a result of the lower efficiency of the second diazo coupling as demonstrated from the combustion data, leading from polymer **20** to **23a,b**, as compared to the direct insertion of the carbamates of **4c**, **8c**, **11c**, **14c**, and **17c**. In related work using diazonium salt coupling reactions for surface modification, we have shown that only one diazonium residue couples with activated aromatic residues.⁴⁵

Treatment of modified polymers **23a,b** with hydrogen peroxide in an analogous manner to that described above then gave

polymers **24a,b** in which the peroxide had been adsorbed, which was quantified by iodometric titration (Table 5) at levels of 0.69×10^{14} and 1.2×10^{14} molecules $\cdot\text{cm}^{-2}$, respectively. It was found that polymers **24a** and **24b** bind 6 to 7 molecules of H_2O_2 after 1 h and 14 to 15 molecules after 24 h of immersion in H_2O_2 solution, with only a 17% loss of activity for **24a** and a 39% loss of activity in **24b** after 96 h (Tables 5 and 7). Thus, polymer **24a** is better at retaining H_2O_2 than is **24b** and significantly better than simple urethane systems **10**, **13**, **16**, and **19**.

Overall, the loading levels were found to be best for **13**, **16**, and **24b** ($[\text{H}_2\text{O}_2]$ over the range of 1.0–1.5 mmol/g), where H bonding leads to strong adduct formation; moreover, these same materials exhibited the best levels of stability, retaining high levels of activity after 96 h (loss of activity in the range of 35–50%). The most stable adduct was clearly **24a**, which showed only a 17% loss of activity after 96 h.

To access the biocidal properties of these materials, assay against five representative gram-positive (*Staphylococcus aureus* and *Bacillus thuringiensis*) and gram-negative (*Escherichia coli*, *Xanthomonas campestris*, and *Pseudomonas syringae*) bacteria was made (Table 7). With one exception, all of the peroxide-modified polymers showed activity against all microorganisms, although not all at the same level of potency. Polymers **6**, **10**, and **13**, all containing urea residues, gave very good activity against all strains of bacteria, especially against the more resistant *S. aureus*. Whereas thiourea-containing polymer **16** showed extremely high activity against all strains of gram-negative and gram-positive bacteria, the other thiourea-containing polymer, **19**, generally showed much lower bioactivity. The antimicrobial activity of both **24a** and **24b** against *E. coli* was good, but in the case of other bacterial strains, uramil **24a** showed less activity than thiouramil

24b. The particularly low activity of **21**, which contains no groups capable of hydrogen bonding to hydrogen peroxide, is noteworthy. Moreover, not all organisms were equally responsive, with *E. coli* and *S. aureus* being the most susceptible and *X. campestris* being the least susceptible to bactericidal action by hydrogen peroxide.

The most active polymers are thiocarbamates **16** and **24b**, although carbamates **6**, **10**, and **24a** also displayed a high level of activity; this activity broadly correlates with H₂O₂ loading (mmol/g), although the lower activity of polymer **13** despite its relatively high loading is noteworthy. In this latter case, the loss of activity after 96 h was only 35%, so the lower activity may reflect the increased stability of this hydrogen peroxide–polymer adduct. Also of interest is that the higher H₂O₂/N ratio for **10** and **13** was not translated into significantly higher bioactivity; however, it is clear that the presence of a carbamate or thiocarbamate is required for activity because polymer **21** displayed activity that was not dissimilar to that of the blank material.

It is noteworthy that hydrogen peroxide has been reported to be lethal to a variety of spore-forming and non-spore-forming bacteria at concentrations of 3–15% over 30–60 min exposure periods,⁶⁴ whereas its bacteriostatic MIC value against a panel of bacteria has been found to be 0.15 mmol/L and it exhibits sporicidal activity at pH 5 with a killing time of 3 h.⁶⁵ Although the killing time has not been probed in our work, we note that our bioassay involves a 16 h incubation and it is therefore likely that the killing times of our modified polymers will be similar to those observed in solution. Moreover, the bactericidal activity that is observed at the loading level of these polymers (typically 0.1–1.1 mmol/g^{−1}) is probably achieved as a result of a high local peroxide concentration at the polymer surface as the hydrogen peroxide leaches into solution.

Conclusions

It has been demonstrated that hydrogen peroxide can be loaded effectively onto urea and thiourea-surface-modified polymers, stabilized by hydrogen bonding, and that these materials impart antibacterial activity. Characterization using established protocols including quantitative (combustion analysis and X-ray photoelectron spectroscopy) and qualitative methods (ATR-IR and combustion) confirms the identity and level of surface modification. This concept is potentially generalizable for the targeted delivery of a range of antibacterial agents, and further work in this regard will be reported in due course.

Experimental Section

Synthesis. The syntheses of 4-bromomethylbenzophenone,⁴⁰ *N*-(2-(4-diazo(phenyl)methyl)benzoyloxy)-ethyl)-*N*-ethylaniline, uramil, uramil diazonium salt,^{31,32} 2-thiouramil,^{66,67} 2-thiouramil diazonium salt,^{31,32} and diazo **3**⁴⁰ have been previously reported.

General Method I: H-Acid Test for the Presence of the Diazonium Functionality. H acid (4-amino-5-hydroxynaphthalene-2,7-disulfonic acid) was dissolved in water to achieve an opaque beige solution (approximately 1:2 H acid/water v/v). The diazonium compound was added to the H-acid solution, which was then agitated to ensure thorough mixing. The solution was left for 5 min for the color to develop. A positive H-acid test was observed as a significant color change from beige to purple resulting from the surface reaction of the diazonium compound.

General Method II: Functionalization of Polystyrene Beads. The appropriate diaryldiazomethane(IV) (100 mg) was dissolved in ether or THF (5 mL) in a 50 mL flask. The Amberlite XAD-4 polymer (1 g) was then placed in the solution, and the solvent was removed in vacuo at room temperature. This sample was carefully heated in an oil bath at 120 °C. When no pink color, due to diaryldiazomethane, was observable in the appropriate sample, heating was stopped and the sample was washed with excess acetone.

General Method III: Coupling of the Diazo Compound on Modified Polystyrene Beads. Each portion of functionalized Amberlite was then placed in a separate flask, and a suspension of diazonium salt in water was added and left to stand overnight. Each sample was then carefully washed with water and acetone by filtration through a sintered funnel. This process was repeated until no color was seen to be washed out, and the final samples were kept for analysis.

General Method IV: Bacteria-Seeded Agar Plate Preparation. The microorganism was washed off a nutrient slope with sterile water (5 mL). The specific bacterial solution was diluted to 1:50 with sterile water and was added to cooled molten agar in a 1:100 ratio (bacterial solution (1:50): agar, v/v). The inoculated agar (17 mL) was pipetted into empty Petri dishes (90 mm), which were then swirled to ensure even agar thickness. The Petri dishes were allowed to set and were refrigerated until needed.

General Method V: Polymer Bioassay. Using a sterile method, 6- or 10-mm-diameter circles were punched in the agar seeded with bacteria (prepared according to method IV). The inner agar was removed to produce empty wells. The test polymer was accurately weighed (10 mg) and added to prepunched wells of the seeded agar plates. The well was then sealed with another 100 μ L of molten agar so that a uniform layer of agar was produced. The agar plates were covered and incubated for 18–24 h to encourage bacterial growth. The diameter of the antimicrobial clear zones around each well were measured and recorded per test compound. A blank polymer sample was taken as a reference.

General Method VI: Hydrogen Peroxide Loading on the Polymer Samples. A sample of the required polymer (10–30 mg) was suspended in 50% aqueous hydrogen peroxide for 1 h, and the polymer was collected by filtration and washed with 1–1.5 L of water, collected, and treated with a solution of 20 mL of 10% potassium iodide in 10 mL of aqueous acetic acid. After standing for 5 min, a few milliliters of aqueous starch solution was added and the suspension was left to stand for 1 h. The resulting dark-blue solution was titrated with 0.01 M sodium thiosulfate until a colorless end point that typically requires 1.0–5.0 mL of solution was reached. The mixture was left for another hour, and any further blue coloration of the solution was titrated with sodium thiosulfate.

General Method VII: Stability Testing of Hydrogen Peroxide on the Polymer Samples. A sample of the functionalized polymer was suspended in 50% aqueous hydrogen peroxide for 18–24 h. The polymer was collected by filtration and washed with 1–1.5 L of water, collected in a stoppered vial, and stored at room temperature in a darkened cupboard. At regular time intervals, 10 mg samples were collected and tested for hydrogen peroxide loading using general method VI.

General Method VIII: Method for Testing the Regenerability of Hydrogen Peroxide-Functionalized Polymers. A sample of the required polymer (0.05 g) was suspended in 50% aqueous hydrogen peroxide (2 mL) for 18 h. The polymer was collected by filtration, washed with 1–1.5 L of water, and then collected and treated with a solution of 10% potassium iodide in acetic acid and water. After 5 min of standing, a 1% aqueous starch solution was added and the suspension was left to stand for 1 h. A positive test for hydrogen peroxide was the formation of a dark-blue solution. This mixture was then quenched with sodium thiosulfate until colorless, and the polymer was filtered and washed with 1–1.5 L of water. The polymer was then resuspended

(64) Wardle, M. D.; Renninger, G. M. *Appl. Microbiol.* **1975**, *30*, 710–711.

(65) Baldry, M. G. C. *J. Appl. Bacteriol.* **1983**, *54*, 417–423.

(66) Mario, R.; Piero, P.; Silvio, C.; Francesco, C. *Gazz. Chim. Ital.* **1954**, *84*, 781–791.

(67) Johnson, T. B.; Shepard, N. A. *J. Am. Chem. Soc.* **1913**, *35*, 994–1007.

in fresh 50% aqueous hydrogen peroxide (2 mL), and the cycle was repeated.

4-(Aminoethoxymethyl)benzophenone Hydrochloride 2. To a solution of dry ethanolamine (1.0 g, 16.4 mmol) in dry THF (5 mL) was added sodium hydride (0.7 g, 60% dispersion in mineral oil, 16.4 mmol) in several portions over a period of 5 min. Another aliquot of dry THF (5 mL) was added, and the resulting mixture was stirred for 1 h. To this mixture was added a solution of 4-bromomethyl benzophenone (4.5 g, 16.4 mmol) in dry THF (25 mL). The resulting mixture was allowed to stir for 18 h before being concentrated in vacuo. The residue was partitioned between chloroform and water, and the organic layer was collected, washed with water, and then extracted with 2 M HCl. The combined acidic extractions were washed once with chloroform before being concentrated in vacuo. The residue was dissolved in a 9:1 chloroform/methanol mixture and dried over sodium sulfate before being concentrated in vacuo to yield **2** (2.6 g, 55%) as a pale-yellow solid that was used without further purification.

1-[4-Benzoylbenzyloxyethyl]-3-phenyl Urea 4a. To a stirred suspension of **2** (3.49 g, 12 mmol) in dry DCM (10 mL) was added dry triethylamine (10 mL). After 0.25 h of stirring, a solution of phenyl isocyanate (2.14 g, 18 mmol) in dry DCM (10 mL) was added in one portion. The mixture was stirred for another 16 h and then quenched with water (10 mL). The organic layer was collected and washed sequentially with water, 2 M HCl, and water. The organic layer was dried over MgSO₄ and purified by flash chromatography on silica gel via elution with 2:1 petrol/40:60 ethyl acetate (*R_f* = 0.1) to yield **4a** (3.01 g, 67%) as a white solid; mp 129–132 °C. v_{\max} (film)/cm⁻¹ 3431, 1724, 1658. δ_{H} (DMSO-*d*₆, 200 MHz) 3.39 (q, 2H, *J* = 5.1 Hz, NHCH₂), 3.58 (t, 2H, *J* = 5.0 Hz, OCH₂CH₂), 4.65 (s, 2H, OCH₂Ar), 6.32 (t, 1H, *J* = 5.2 Hz CH₂-NHC(O)), 6.90 (t, 1H, *J* = 7.3 Hz, ArH), 7.24 (t, 2H, *J* = 7.4 Hz, ArH), 7.44 (d, 2H, *J* = 7.6 Hz, ArH), 7.56 (m, 4H, ArH), 7.70 (m, 5H, ArH) 8.63 (s, 1H, C(O)NHAr). δ_{C} (DMSO-*d*₆, 100.6 MHz) 39.9 (CH₂NH), 70.4 (CH₂CH₂O), 72.1 (ArCH₂O), 118.4, 121.9, 128.1, 129.4, 130.4, 130.6, 133.5, 136.9, 138.0, 141.3, 144.4 (all ArC), 156.1 (NHC(O)NH), 196.3 (Ar₂C(O)). *m/z* 375 [M + H]⁺ (5%), 397 [M + Na]⁺ (10%), 433 [M + MeCN + NH₄]⁺ (100%). Found 375.1703; C₂₃H₂₃N₂O₃ requires 375.1709.

1-[2-[4-(Hydrazonophenylmethyl)-benzyloxy]-ethyl]-3-phenyl Urea 4b. A solution of **4a** (0.7 g, 1.9 mmol) in methanol (10 mL) was treated with hydrazine hydrate (1.9 g, 38 mmol). The resulting solution was heated to gentle reflux for 16 h, cooled, and concentrated in vacuo. The residue was partitioned between DCM and water, and the organic layer was collected, washed with water, and dried over MgSO₄. Concentration in vacuo yielded **4b** (0.7 g, 99%) as a thick, cloudy white oil. v_{\max} (film)/cm⁻¹ 3435, 1734. δ_{H} (CDCl₃, 200 MHz) 3.47 (m, 2H, NHCH₂), 3.61 (m, 2H, OCH₂CH₂), 4.43 and 4.52 (2s, 2H, ArCH₂O), 5.49 (s, 2H, NNH₂), 6.55 (m, 1H, CH₂NHC(O)), 6.99 (m, 1H, ArH), 7.22 (m, 7H, ArH), 7.46 (m, 6H, ArH), 7.80 (m, 1H, ArNHC(O)). *m/z* 389 [M + H]⁺ (15%), 447 [M + MeCN + NH₄]⁺ (100%). Found 389.1973; C₂₃H₂₅N₄O₂ requires 389.1978.

1-[2-[4-(Diazophenylmethyl)benzyloxy]ethyl]-3-phenyl Urea 4c. To a vigorously stirred mixture of yellow mercury oxide (0.18 g, 0.8 mmol), sodium sulfate (0.14 g, 1 mmol) and sat. KOH in ethanol (1 mL) was added a solution of **4b** (0.27 g, 0.7 mmol) in THF (10 mL). The mixture was stirred for 16 h in the dark and then filtered through a pad of Celite. The filtrate was collected and concentrated in vacuo to yield **4c** (0.27 g, 100%) as a dark-red solid, mp 111–115 °C, that decolors at 136 °C. v_{\max} (film)/cm⁻¹ 3344, 2042, 1658. δ_{H} (CDCl₃, 200 MHz) 3.43 (m, 2H, CH₂NH), 3.69 (m, 2H, CH₂O), 4.49 (s, 2H, ArCH₂O), 5.80 (bs, 1H, CH₂NHC(O)), 7.02 (m, 1H, ArH), 7.41–7.44 (m, 13H, ArH), 7.44 (s, 1H, ArNHC(O)). δ_{C} (CDCl₃, 100.6 MHz) 40.3 (CH₂NH), 62.4 (CH₂CH₂O), 68.0 (ArCH₂O), 120.3 (ArC), 123.2 (ArC), 125.0, 125.8, 126.5, 126.7 (ArC), 128.9, 129.1, 129.2, 129.3 (ArC), 135.0, 138.9 (ArC), 156.5 (NHC(O)NH). *m/z* 445 [M + MeCN + NH₄]⁺ (100%).

4-(Aminoethoxymethyl)benzophenone 2 and 4-((2-Hydroxyethylamino)methyl)phenyl-(phenyl)methanone 7. To a solution of dry ethanolamine (2.21 g, 36.23 mmol) in dry THF (10 mL) was added sodium hydride (1.22 g, 60% dispersion in mineral oil, 36.23 mmol) in several portions over a period of 5 min. Another aliquot of dry THF (10 mL) was added, and the resulting mixture was stirred for 1 h. To this mixture was added a solution of 4-bromomethyl benzophenone **1** (10 g, 36.23 mmol) in dry THF (40 mL). The resulting mixture was allowed to stir for 18 h before being concentrated in vacuo. The residue was partitioned between chloroform and water, and the organic layer collected and then extracted with 2 M HCl. The combined acidic extractions were washed once with chloroform and concentrated in vacuo. The residue was dissolved in a 9:1 chloroform/methanol mixture and dried over sodium sulfate before being concentrated in vacuo to yield the product mixture of **2** and **7** (5.1 g) as a white solid. v_{\max} (film)/cm⁻¹ 1652, 1593, 1461, 1377. δ_{H} (CDCl₃, 400 MHz) 3.1–3.2 (m, 2H, CH₂NH₂ and CH₂N), 3.9–4.0 (m, 2H, CH₂OH and CH₂O), 4.30–4.31 (s, 2H, ArCH₂N), 4.70 (s, 2H, ArCH₂O), 7.4–7.9 (m, 9H, ArH), 10.2 (bs, 2H, NH and/or OH). δ_{C} (CDCl₃, 100.6 MHz) 45.3 (CH₂N), 50.7 (ArCH₂N), 55.0 (CH₂N), 57.6 (CH₂O), 65.8 (CH₂O), 72.8 (ArCH₂O), 126.6, 127.4, 128.2, 128.3, 128.4, 128.5, 129.0, 130.0, 130.1, 130.3, 130.5, 130.6, 130.8, 131.6, 132.2, 132.5, 132.8, 133.0, 133.4, 136.7, 137.1, 137.4, 138.5, 139.1, 141.9, 195.6, 196.3. *m/z* 256.2 [M + H]⁺, 314.2 [M + CH₃CN + NH₄]⁺, 254.1 [M – H]⁺. HRMS: [M + H]⁺ measured 256.1334, calculated 256.1332 for [C₁₆H₁₇NO₂ + Na]⁺.

1-(2-(4-Benzoylbenzyloxy)ethyl)-3-phenylurea 4a, 1-(4-Benzoylbenzyl)-1-(2-hydroxyethyl)-3-phenylurea 8a, and 2-(1-(4-Benzoylbenzyl)-3-phenylureido)ethylphenylcarbamate 11a. To a stirred suspension of **2** and **7** (5 g, 19.60 mmol) in dry DCM (20 mL) was added dry triethylamine (3.96 g, 39.22 mmol). After 15 min of stirring, a solution of phenyl isocyanate (3.3 g, 27.5 mmol) in dry DCM (30 mL) was added in one portion. The mixture was stirred for another 16 h and then quenched with water (10 mL). The organic layer was collected and washed sequentially with water, 2 M HCl, and water. The organic layer was dried over Na₂SO₄ and purified by flash chromatography on silica gel via elution with 2:1 petrol/40:60 ethyl acetate to yield **4a** (0.9 g, 18%), **8a** (1.2 g, 24%), and **11a** (2.75 g, 55%) as white solids with mp's of 181, 178 and 160 °C respectively.

Data for **4a** is the same as that given above.

Data for **8a**: v_{\max} (film)/cm⁻¹ 2926, 2850, 1654. δ_{H} (CDCl₃, 400 MHz) 3.67 (t, 2H, *J* 4.0 Hz, NHCH₂), 4.35 (t, 2H, *J* 4.1 Hz, OCH₂), 4.82 (s, 2H, NCH₂Ar), 6.94 (t, 1H, ArH), 7.22–7.25 (m, 2H, ArH), 7.41–7.47 (m, 4H, ArH), 7.53–7.57 (m, 2H, ArH), 7.65–7.74 (m, 5H, ArH), 8.53 (CONHCH₂), 9.65 (s, 1H, CONHAr). δ_{C} (DMSO-*d*₆, 400 MHz) 46.4, 50.8, 63.2, 121.1, 122.9, 123.4, 127.9, 129.4, 129.6, 130.4, 131.9, 133.5. *m/z* (ES⁺) 433.2 [M + CH₃CN + NH₄]⁺; *m/z* (ES⁻) 373.2 [M – H]⁺. The structure was further authenticated by X-ray crystallography (Figure 1).

Data for **11a**: v_{\max} (film)/cm⁻¹ 3396, 3210, 1724, 1647. δ_{H} (CDCl₃, 400 MHz) 3.67 (t, 2H, *J* 5.5 Hz, NHCH₂), 4.25 (t, 2H, *J* 5.5 Hz, OCH₂), 4.87 (s, 2H, NCH₂Ar), 6.93–7.00 (m, 2H, ArH), 7.21–7.28 (m, 4H, ArH), 7.43–7.56 (m, 8H, ArH), 7.64–7.74 (m, 5H, ArH), 8.52 (s, 1H, NCONHAr), 9.67 (s, 1H, OCONHAr). δ_{C} (CDCl₃, 100.6 MHz) 46.40 (NCH₂), 50.82 (ArCH₂N), 63.20 (OCH₂CH₂N), 119.25, 121.09 (ArCH), 122.95, 123.36 (ArCH), 127.94, 129.11, 129.40, 129.58, 130.41, 130.88, 133.46 (ArCH), 136.56, 137.97 (ArC(C=O)), 141.07, 144.71 141.27 (ArCNHR), 154.27 (OCONH), 156.12 (NCONH), 196.26 (C=O). *m/z* (ESI⁺) 552.2 [M + CH₃CN + NH₄]⁺. HRMS [M + Na]⁺ measured 516.1898, calculated 516.1894 for [C₃₀H₂₇N₃O₄ + Na]⁺. The structure was also determined by X-ray crystallography (Figure 2).

1-(2-(4-(Hydrazono(phenyl)methyl)benzyloxy)ethyl)-3-phenylurea 4b, 1-(4-(Hydrazono(phenyl)methyl)benzyl)-1-(2-hydroxyethyl)-3-phenylurea 8b, and 2-(1-(4-Hydrazono(phenyl)methyl)benzyl)-3-phenylureido)ethyl Phenyl Carbamate 11b. A solution of ketone **8a** (1 equiv) in methanol (10 mL) was treated

with hydrazine hydrate (20 equiv). The resulting solution was heated to gentle reflux for 16 h, cooled, and concentrated in vacuo. The residue was partitioned between DCM and water, and the organic layer was collected, washed with water, and dried over MgSO_4 . Concentration in vacuo yielded hydrazone (a mixture of syn- and anti- isomers) **8b** (2.2 g, 89%) as a thick, cloudy white oil.

Data for **8b**: δ_{H} (CDCl_3 , 400 MHz) 3.44–3.70 (m, 4H, $\text{NCH}_2\text{-CH}_2$), 4.48 and 4.65 (2s, 2H total, NC H_2Ar), 5.20–5.48 (m, 2H, NNH_2), 6.84 (s, 1H, NHCON), 6.98–7.09 (m, 2H, ArH), 7.10–7.34 (m, 18H, ArH), 7.36–7.69 (9H, m, ArH). m/z (ESI+) 389.2 $[\text{M} + \text{H}]^+$, (ESI+) 387.2 $[\text{M} - \text{H}]^-$, 447.3 $[\text{M} + \text{CH}_3\text{CN} + \text{NH}_4]^+$. HRMS $[\text{M} + \text{K} + \text{H}]^+$ measured 429.2277, calculated 429.2261 for $[\text{C}_{23}\text{H}_{24}\text{N}_4\text{O}_2 + \text{K} + \text{H}]$.

Data of **4b**: The same procedure was repeated with ketone **4a** to give hydrazone **4b** as a thick oil. m/z (ESI+) 387.2 $[\text{M} - \text{H}]^-$. Data are the same as given above.

Data for **11b**: The same procedure was repeated with ketone **11b** to give hydrazone **11b** as a thick oil (1.24 g, 73%). δ_{H} (CDCl_3 , 400 MHz) 3.78–4.0 and 4.0–4.45 (m, 4H, $\text{NHCH}_2\text{CH}_2\text{O}$), 4.75 and 4.85 (2s, 2H, OCH_2Ar), 5.90 (2s, 2H, NNH_2), 6.98–7.19 (m, 2H, ArH), 7.10–7.54 (m, 18H, ArH), 7.59–7.93 (H, m, ArH), 7.95–8.93 (2H, s, NHCONH). m/z (ESI+) 566.3 $[\text{M} + \text{CH}_3\text{CN} + \text{NH}_4]^+$.

1-(2-(4-(Diazophenyl)methyl)benzyloxy)ethyl-3-phenylurea 4c, **1-(4-(Diazophenyl)methyl)benzyl-1-(2-hydroxyethyl)-3-phenylurea 8c**, and **2-(1-(4-(Diazophenyl)benzyl)-3-phenylureido)ethylphenylcarbamate 11c**. To a vigorously stirred mixture of yellow mercury oxide (1.14 equiv), sodium sulfate (1.4 equiv), and sat. KOH in ethanol (1 mL) was added a solution of hydrazone **8b** (1 equiv) in THF (10 mL). The mixture was stirred for 16 h in the dark and then filtered through a pad of Celite. The filtrate was collected and concentrated in vacuo to yield diazo **8c** (1.88 g, 91%) as a pink solid, which was used without further purification. The mp is 111–115 °C, and decolorization occurs at 136 °C.

Data for **8c**: δ_{H} (CDCl_3 , 400 MHz) 3.43 (m, 2H, NCH_2CH_2), 3.62 (m, 2H, NCH_2CH_2), 4.58 (s, 2H, NCH_2Ar), 5.21 (s, 1H, OHCH_2), 6.71–7.58 (m, 14H, ArH), 7.61–7.89 (s, 1H, CONHAr). m/z (ESI+): 445.2 $[\text{M} + \text{CH}_3\text{CN} + \text{NH}_4]^+$, (ESI–): 385.2 $[\text{M} - \text{H}]^+$.

Data of **4c**: The same procedure was repeated with hydrazone **4b**, and dark-pink diazo **4c** was obtained, which was used without further purification. m/z (ESI+) 445.2 $[\text{M} + \text{CH}_3\text{CN} + \text{NH}_4]^+$.

Data for **11c**: The same procedure was repeated with hydrazone **11b**, and dark-pink diazo **11c** was obtained (0.98 g, 89%), which was used without further purification. v_{max} (film)/ cm^{-1} 2040, 1769, 1652. m/z (ESI+) 566.3 $[\text{M} + \text{CH}_3\text{CN} + \text{NH}_4]^+$.

1-(2-(4-Benzoyl-benzyloxy)ethyl)-3-phenylthiourea 14a and **1-(4-Benzoylbenzyl)-1-(2-hydroxyethyl)-3-phenylthiourea 17a**. To a stirred suspension of **2** and **7** (1 equiv) in dry DCM (20 mL) was added dry triethylamine (2 equiv). After 15 min of stirring, a solution of phenyl isocyanate (1.2 equiv) in dry DCM (30 mL) was added in one portion. The mixture was stirred for another 16 h and then quenched with water (10 mL). The organic layer was collected and washed sequentially with water, 2 M HCl, and water. The organic layer was dried over Na_2SO_4 and purified by flash chromatography on silica gel via elution with 2:1 petrol/40:60 ethyl acetate to yield **14a** (2.83 g, 57%) and **17a** (1.84 g, 37%) as a white solids.

Data for **14a**: v_{max} (film)/ cm^{-1} 3290, 1655, 1315. δ_{H} (CDCl_3 , 400 MHz) 3.74 (t, 2H, $J = 5.0$ Hz, NHCH_2), 3.91 (m, 2H, OCH_2), 4.55 (s, 2H, OCH_2Ar), 6.58 (s, 1H, CSNHAr), 7.19 (m, 1H, ArH), 7.21–7.29 (m, 4H, ArH), 7.35–7.39 (m, 2H, ArH), 7.47–7.51 (m, 2H, ArH), 7.58–7.62 (m, 1H, ArH), 7.73–7.79 (m, 4H, ArH), 8.12 (s, 1H, CSNHAr). δ_{C} (CDCl_3 , 100.6 MHz) 45.14 (NCH_2CH_2), 60.40 (OCH_2), 72.51 (ArCH_2O), 125.03 (ArCH), 127.13 (ArCH), 128.31, 130.01, 130.14, 130.29, (ArCH), 132.53 (ArCH), 136.99 (ArCNHR), 137.50 ($\text{ArC}(\text{C}=\text{O})$), 142.37 (ArCCH_2), 171.18 (NHCSNH), 196.30 ($\text{C}=\text{O}$). m/z (ESI+) 449.2 $[\text{M} + \text{CH}_3\text{CN} + \text{NH}_4]^+$, (ESI–) 389.1 $[\text{M} - \text{H}]^+$. HRMS

$[\text{M} + \text{Na}]^+$ measured 413.1288, calculated 413.1294 for $[\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}_2\text{S} + \text{Na}]^+$.

Data for **17a**: v_{max} (film)/ cm^{-1} 3276, 1654, 1598, 1562, 1317, 1212, 1059. δ_{H} (CDCl_3 , 400 MHz) 3.75 (m, 2H, NHCH_2), 3.85 (m, 2H, OCH_2), 5.32 (s, 2H, NCH_2Ar), 7.14–7.17 (m, 1H, ArH), 7.27 (s, 1H, OH), 7.32–7.36 (m, 4H, ArH), 7.43–7.51 (m, 4H, ArH), 7.58–7.63 (m, 1H, ArH), 7.79–7.81 (m, 4H, ArH), 9.46 (s, 1H, CSNHAr). δ_{C} (CDCl_3 , 100.6 MHz) 52.33 (OCH_2), 55.14 ($\text{OCH}_2\text{CH}_2\text{N}$), 61.51 (ArCH_2N), 124.07 (ArCH), 125.00 (ArCH), 127.35 (ArCH), 128.35, 128.59, 130.07, 130.59 (ArCH), 132.60 (ArCH), 136.89 ($\text{ArC}(\text{C}=\text{O})$), 137.40 (ArCNHR), 140.23 (ArCCH_2), 184.52 (NHCSNH), 196.50 ($\text{C}=\text{O}$). m/z (ESI+) 449.2 $[\text{M} + \text{CH}_3\text{CN} + \text{NH}_4]^+$, (ESI–) 389.1 $[\text{M} - \text{H}]^+$. HRMS $[\text{M} + \text{H}]^+$ measured 391.1470, calculated 391.1475 for $[\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}_2\text{S} + \text{H}]^+$. The structure was authenticated by X-ray crystallography (Figure 3).

1-(2-(4-(Hydrazono-phenyl-methyl)-benzyloxy)-ethyl)-3-phenylthiourea 14b and **1-(4-(Hydrazono(phenyl)methyl)-benzyl)-1-(2-hydroxyethyl)-3-phenyl Thiourea 17b**. A solution of ketone **14a** (1 equiv) in methanol (10 mL) was treated with hydrazine hydrate (20 equiv). The resulting solution was heated to gentle reflux for 16 h, cooled, and concentrated in vacuo. The residue was partitioned between DCM and water, and the organic layer was collected, washed with water, and dried over MgSO_4 . Concentration in vacuo yielded hydrazone **14b** (a mixture of syn- and anti- isomers) (0.85 g, 85%) as a thick, cloudy white oil.

Data for **14b**: v_{max} (film)/ cm^{-1} 3310, 1533, 1091. δ_{H} (CDCl_3 , 400 MHz) 3.58 and 3.76 (2t, 2H, $J = 5.0$ and 4.9 Hz, $\text{NHCH}_2\text{-CH}_2$), 3.40–3.80 (m, 2H, NHCH_2), 4.36 and 4.63 (2s, 2H, OCH_2Ar), 5.45 (m, 2H, NNH_2), 6.57 (m, 1H, CH_2NHCS), 7.07–7.09 (m, 2H, ArH), 7.16–7.38 (m, 18H, ArH), 7.39–7.56 (m, 8H, ArH), 7.79 (m, 1H, CSNHAr). m/z (ESI+) 463.2 $[\text{M} + \text{CH}_3\text{CN} + \text{NH}_4]^+$, (ESI–) 403.2 $[\text{M} - \text{H}]^+$. HRMS $[\text{M} + \text{Na}]^+$ measured 427.1557, calculated 427.1563 for $[\text{C}_{23}\text{H}_{24}\text{N}_4\text{OS} + \text{Na}]^+$.

Data for **17b**: The same procedure was repeated with ketone **17a** and hydrazone as thick, cloudy oil **17b** was obtained (1.29 g, 86%). v_{max} (film)/ cm^{-1} 3287, 1601, 1497, 1444, 1052. δ_{H} (CDCl_3 , 400 MHz) 2.32 (s, 1H, NH), 2.80 and 2.85 (2m, 2H, NCH_2), 3.60 and 3.75 (2m, 2H, CH_2OH), 3.80 and 3.95 (2s, 2H, NCH_2Ar), 5.37 (s, 1H, OH), 5.43 (m, 2H, NNH_2), 6.67–7.20 (m, 2H, ArH), 7.21–7.48 (m, 18H, ArH), 7.49–7.71 (m, 8H, ArH). δ_{C} (CDCl_3 , 100.6 MHz) 50.3 and 50.6 (OCH_2), 52.9 and 53.2 ($\text{OCH}_2\text{CH}_2\text{N}$), 60.7 and 60.9 (ArCH_2N), 126.5, 126.6, 128.1, 128.8, 129.0, 129.2, 129.3, 129.4. m/z (ESI+) 404.2 $[\text{M} + \text{H}]^+$, (ESI–) 403.2 $[\text{M} - \text{H}]^+$. HRMS $[\text{M}]^+$ measured 403.1596, calculated 403.1598 for $[\text{C}_{23}\text{H}_{24}\text{N}_3\text{OS}]^+$.

1-(2-(4-(Diazophenyl)methyl)benzyloxy)ethyl-3-phenylthiourea 14c and **1-(4-(Diazophenyl)benzyl)-1-(2-hydroxyethyl)-3-phenylthiourea 17c**. To a vigorously stirred mixture of manganese dioxide (3.0 equiv), sodium sulfate (1.4 equiv), and sat. KOH in ethanol (1.04 equiv) was added a solution of hydrazone **14b** (1 equiv) in THF (10 mL). The mixture was stirred for 16 h in the dark and then filtered through a pad of Celite. The filtrate was collected and concentrated in vacuo to yield diazo **14c** (0.73 g, 91%) as a dark-pink solid with a mp of 112–114 °C that decolors at 140 °C.

Data for **14c**: v_{max} (film)/ cm^{-1} 3382, 2038, 1655, 1593, 1097. δ_{H} (CDCl_3 , 400 MHz) 3.63 (m, 2H, NHCH_2), 3.82 (m, 2H, CH_2O), 4.52 (s, 2H, OCH_2Ar), 6.62–6.97 (s, 1H, CH_2NHCS), 7.02–7.32 (m, 14H, ArH), 7.70–7.85 (m, 1H, CSNHAr). m/z (ESI+) 462.2 $[\text{M} + \text{CH}_3\text{CN} + \text{NH}_4]^+$, (ESI–) 401.2 $[\text{M} - \text{H}]^+$. HRMS $[\text{M}]^+$ measured 401.1434, calculated 401.1442 for $[\text{C}_{23}\text{H}_{22}\text{N}_4\text{OS}]^+$.

Data for **17c**: The same procedure was repeated with hydrazone **17b**, and red solid diazo **17c** was yielded (1.0 g, 83%) with a mp of 124 °C and decolorization at 140 °C. v_{max} (film)/ cm^{-1} 3301, 2038, 1593, 1493, 1053. δ_{H} (CDCl_3 , 400 MHz) 2.83 (m, 2H, CH_2OH), 3.64 (m, 2H, NCH_2), 3.82 (s, 2H, NCH_2Ar), 6.63–7.08 (s, 1H, OH), 7.10–7.45 (m, 14H, ArH), 7.70–7.83 (m, 1H, CSNHAr). m/z (ESI+) 441.3 $[\text{M} + \text{K}]^+$.

X-ray Structure Determination. Single-crystal diffraction data for **8a**, **11a**, and **17a** were collected at 150(2) K with an Oxford Cryosystems Cryostream N2 open-flow cooling device.⁶⁸ For **8a** and **17a**, data were collected on an Enraf-Nonius Kappa CCD diffractometer (Mo K α radiation (λ = 0.71073 Å) and processed using the DENZO-SMN package,⁶⁹ including inter-frame scaling (which was carried out using Scalepack within DENZO-SMN). Data for **11a** were initially collected similarly but were found to be of poor quality with the structure exhibiting extensive disorder. The cause of the difficulties was unclear and modulation and twinning were thought to be possible, so in order to resolve the reflections better, data were collected with synchrotron radiation using beamline I19 (EH1) at the Diamond Light Source. Although the data were examined for satellite reflections, none were found and the data were processed using CrystalClear.⁷⁰ All three structures were solved using SIR92,⁷¹ and refinement was carried out using full-matrix least squares within the CRYSTALS suite⁷² on either F² or F (**11a** only). Except for one phenyl ring in **11a** (where the close proximity of the components destabilized the refinement), all non-hydrogen atoms were refined with anisotropic displacement parameters at same distance, with thermal and vibrational restraints to ensure that the geometry and ADPs of disordered components remained sensible.

(68) Cosier, J.; Glazer, A. M. *J. Appl. Crystallogr.* **1986**, *19*, 105–107.

(69) Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307–326.

(70) Rigaku Americas and Rigaku Corporation. *CrystalClear*, version 2.0; Rigaku Americas: The Woodlands, TX, 2009.

(71) Altomare, A.; Cascarano, G.; Giacovazzo, G.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Crystallogr.* **1994**, *27*, 435.

(72) Betteridge, P. W.; Carruthers, J. R.; Cooper, R. I.; Prout, K.; Watkin, D. J. *J. Appl. Crystallogr.* **2003**, *36*, 1487–1487.

The disorder in **11a** was extensive with 28 out of 37 non-hydrogen atoms significantly affected. Although this leads to potential ambiguity in the atom types, from the synthesis pathway and the electron density there seems little doubt that the connectivity is correct. Hydrogen atoms on nitrogen atoms were generally located in the difference map. Together with the other hydrogen atoms, their positions and isotropic displacement parameters were refined using restraints prior to inclusion in the model with riding constraints. The exception to this was structure **11a**, for which the N–H hydrogen atoms were not visible in the difference map because of the disorder, that was therefore added geometrically on the basis of the chemistry. Full crystallographic data for all structures have been deposited with the Cambridge Crystallographic Data Centre, CCDC 784484 – 784486. Copies of these data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Supporting Information Available: Compound and polymer characterization data. This material is available free of charge via the Internet at <http://pubs.acs.org>.